Remarks

Claims 1-14, 27-41, and 43-65 are pending. Claims 6-14, 27-41 and 43-52 have been withdrawn from consideration by the Examiner as directed to a nonelected invention. Claims 1-5 and 53-65 are under examination as they read on an isolated nucleotide sequence of SEQ ID NO:1, encoding a polypeptide of SEQ ID NO:4, vectors, host cells and methods of producing the polypeptide.

Rule 131 Declaration

The Examiner alleges that the Declaration submitted under 37 C.F.R. 1.131 on January 14, 2003 is defective because it contains alterations that are non-initialed and non-dated. The Examiner further alleges that two copies of the declaration are found, one for each inventor, and that one declaration has alterations an another does not. Applicants respectfully submit that this is due to a mistaken belief by Applicants' representative that it was acceptable for Applicant Starling to correct an error with his citizenship and address by striking through the incorrect citizenship and address and handwriting his corrections.

Submitted herewith is a Rule 131 Declaration (Appendices A and B) which is substantively the same as the Rule 131 Declaration submitted on January 14, 2003 but includes the correct citizenship and address of each applicant, and has been amended to reflect that it is being submitted in response to the outstanding Final Office Action. Both Applicants have reviewed the Rule 131 Declaration submitted herewith and signed their names thereto.

Applicants respectfully submit that this Declaration is acceptable and applicable to the outstanding grounds of rejection as set forth below.

Section 112, second paragraph, Rejections (Definiteness)

The Examiner has maintained the rejection that "hybridizes under stringent conditions" (which now appears in Claim 54) is ambiguous. The Examiner asserts that the specification

discloses merely general parameters for calculating such conditions, but that it is unclear which conditions are actually claimed. This rejection is respectfully traversed.

As stated in Applicants' Response dated December 4, 2002, pages 31-32 of the instant specification do not merely set forth general parameters, but rather specific conditions which are readily recognized by those of skill in the art to result in a clearly and readily detectable hybridization signal. For example, as set forth on page 31, lines 5 and 10, stringent salt conditions are desirably less than about 250 mM NaCl and 25 mM sodium citrate and stringent temperature conditions are at least about 42ºC, respectively.

Section 112, second paragraph, does not require that each term in a claim be substituted for that which it defines. Rather, it requires that each claim be definite. In the present case, one skilled in the art may readily look to the present specification for guidance to determine the scope of "stringent conditions" within the context of the present claims in order to determine under which conditions a clear hybridization signal may be obtained. Applicants respectfully submit that it is not necessary to recite all of those conditions set forth in the instant specification in the present claims in place of "stringent conditions". As the claims are to be read in view of the teachings of the specification, it follows that if replacing "stringent conditions" with the conditions set forth in the specification is definite as required by Section 112, second paragraph, then the use of "stringent conditions" read in view of the specification must also be definite.

Accordingly, Applicants submit that withdrawal of the rejection under Section 112, second paragraph, is appropriate and is respectfully requested.

Section 101 Rejections (Utility)

The Examiner has maintained the rejection of Claim 1-5 and 53-65 under 35 U.S.C. 101 as lacking utility, alleging that the claimed invention is not supported by either a specific and/or substantial asserted utility or a well established utility, essentially for the reasons set forth in the previous Office Action dated June 5, 2002. For the reasons set forth below, this rejection is respectfully traversed.

As stated in Applicants' Response dated December 4, 2002, the standards required of the Patent Office for satisfying Section 101 utility are set forth in the Utility Examination Guidelines (Federal Register, Vol. 66, No. 6, pages 1092-1099, Friday, January 5, 2001). Particularly, in order to satisfy Section 101, the specification must set forth a (1) specific, (2) substantial and (3) credible utility for the claimed invention.

The Invention

The present invention is directed to nucleic acid molecules encoding APEX-1, polynucleotides having certain sequence identity to such nucleic acid molecules, polynucleotides which hybridize to the complement of such nucleic acid molecules, nucleic acid molecules encoding APEX-1 which are labeled with a detectable marker, vectors comprising nucleic acid molecules encoding APEX-1, and host vector systems comprising such vectors.

Specific and Substantial Utility

As set forth in the Utility Examination Guidelines, if Applicants have asserted that the claimed invention is useful for any particular practical purpose (i.e., it has a "specific and substantial utility") and the assertion would be considered credible by a person of ordinary skill in the art, then the utility requirement of Section 101 is satisfied.

The present specification clearly sets forth both specific and substantial utility for the claimed invention. For example, as pointed out by the Examiner, APEX or an agonist thereof may be administered to treat any number of known disorders, including inflammatory, cancer and immune disorders.

It is well established that nucleic acids and proteins encoded thereby, such as APEX, which are shown to be expressed in various tissues, may be biological targets for the treatment of disease states associated with such tissues. Indeed, the patent literature is replete with such examples. Accordingly, Applicants respectfully submit that the present invention clearly has specific and

substantial utility. The use of the claimed molecules as biological targets alone satisfies this requirement.

Credible Utility

With respect to the credible utility requirement, the present specification states repeatedly that the claimed invention shows homology to a well-characterized class, namely the CD2 subfamily. The Examiner states that the instant situation is directly analogous to that which was addressed in Brenner v. Manson 148, USPQ 689 (1966) (hereinafter "Brenner"). In the outstanding office action, the Examiner maintains reliance on Brenner, stating that "Congress intended that no patent be granted on a chemical compound whose sole "utility" consists of its potential role as an object of use-testing". Applicants arguments in reply are as set forth in the Response dated December 4, 2002 and hereinbelow.

Applicants respectfully submit that the Examiner's reliance on Brenner is again misplaced. To clarify, Brenner stands for the proposition that a claimed invention must have a practical utility (e.g., must not be useful solely for research purposes) and that utility is not satisfied merely by showing that a compound yielded belongs to a class of compounds which scientists are investigating for possible uses. This is not the case with the present invention.

The present invention is homologous to the CD2 subfamily, which is well-characterized as having utility with respect to leukocyte proliferation, differentiation, migration and activation, and diseases associated therewith (see, for example, "Background of the Invention" of the present application). Accordingly, Applicants are not merely investigating the claimed molecules for possible uses, but rather the claimed molecules have the specific, substantial and credible uses set forth above. Indeed, they have uses similar to those of other members of the CD2 subfamily.

The Examiner asserts that the present rejection is based on the failure to disclose sufficient properties of the protein and/or polynucleotide to support an inference of utility. Additionally, the Examiner states that members of the CD2 subfamily have divergent functions and therefore homology of a compound to members of this class does not support an inference of utility.

However, this is not the standard for Section 101 utility. Under the present law, homology to a molecule with known utility is acceptable for establishing Section 101 utility. Fujikawa v. Wattanasin, 93 F.3d 1559, 1565, 39 USPQ2d 1895, 1900 (Fed. Cir. 1996).

Even assuming, *arguendo*, that the Examiner's characterization of the claimed molecules and the CD2 subfamily is accurate, it is not the law that compounds showing homology to the claimed compounds cannot be classified in a family the members of which may have divergent functions. Applicants' burden is simply to show that the claimed compounds either have demonstrated utility or can be shown to have homology to molecules which have demonstrated utility, as in the present case. In any event, credible utility is even established by the use of the claimed compounds as molecular weight markers (specification page 45, lines 21-23).

Accordingly, as Applicants have asserted specific, substantial and credible utility of the claimed compounds, Applicants respectfully submit that withdrawal of the rejections under Section 101 is appropriate and is respectfully requested.

Section 112, first paragraph, Rejections (Enablement)

The Examiner has rejected Claims 1-5 and 53-65 under 35 U.S.C. 112, first paragraph, as not being enabled. The Examiner states that as the claimed compounds lack utility, one skilled in the art would not know how to make and use the claimed invention. This rejection is respectfully traversed.

Applicants arguments are set forth above as to why the claimed invention has utility under Section 101. Given that, one skilled in the art would readily be able to make and use the claimed compounds as set forth, for example, in the present specification. For example, the claimed compounds may be used in conventional screening assays and may be administered in therapeutically useful compositions. Regarding the Examiner's allegation that the specification fails to teach how to make any isolated nucleic acid molecule encoding APEX-1, Applicants submit that conventional amplification and cloning techniques may readily be used to generate APEX-1. An example is set forth at page 60, lines 25 et seq. of the present specification.

For these reasons, Applicants respectfully submit that withdrawal of the rejections under Section 112, first paragraph, is appropriate and is respectfully requested.

Section 112, first paragraph, Rejections (Written Description)

The Examiner has rejected Claims 1-5 and 53-65 under 35 U.S.C. 112, first paragraph, as lacking written description. The Examiner alleges that the claimed invention is directed to a genus and that there is not a representative number of species provided to support the genus. This rejection is respectfully traversed.

As stated by Applicants in the Response dated December 4, 2002 and reiterated herein, the present specification describes APEX-1 as a molecule having the amino acid sequence set forth in SEQ ID NO:4 and encoded by a nucleic acid having the sequence set forth in SEQ ID NO:1 (Figures 2A, 2B and 5). Therefore, a claim to an APEX-1 molecule is clearly described, no representative number of species need be provided. With respect to the claims directed to variants and polynucleotides which hybridize to complements of APEX-1, Applicants respectfully submit that one of skill in the art, using the extensive teachings in the present specification, would recognize Applicants to be in possession of such molecules. Such molecules are recognized as having the utility set forth with respect for APEX-1, and one of skill in the art will be capable of using the sequences set forth in the present specification to identify variations thereof which are within the scope of the present invention.

For these reasons, withdrawal of the rejection under Section 112, first paragraph, is appropriate and respectfully requested.

Section 102 Rejections

The Examiner has rejected Claims 1, 3-5 and 53-57 under 35 U.S.C. 102(a) as being anticipated by WO 99/63088 ("the '088 publication"). Applicants submitted a Rule 131 Declaration concurrently with a Response dated December 4, 2002, however, the Examiner has noted that said

Declaration is defective. Accordingly, as stated above, Applicants submit herewith a substitute Rule 131 Declaration that corrects the defects noted by the Examiner.

Accordingly, in view of this Rule 131 Declaration, Applicants submit that the '088 publication is not prior art to the present invention, and therefore respectfully submit that the rejection under Section 102(a) is obviated and withdrawal thereof is respectfully requested.

The Examiner has rejected Claims 53-55 under 35 U.S.C. 102(b) as being anticipated by Hillier et al. (GenBank Accession No. H73135) ("Hillier"). The Examiner alleges that Hillier teaches a 436 polynucleotide having 100% polynucleotide identity to the polynucleotide at positions 49-306 of the claimed SEQ ID NO:1. This rejection is respectfully traversed.

In response to this rejection, Applicants previously asserted that Hillier is not a sufficient prior art reference. The description and enabling disclosure requirements of 35 U.S.C. 112, first paragraph, have developed definition through may years of case law and are applied as the minimum qualitative level required for a reference to be effective. In re Hoeksema, 399 F.2d 269, 273, 158 USPQ 596, 600 (CCPA 1969); In re LeGrice, 301 F.2d 929, 936, 133 USPQ 365, 372 (CCPA 1962). Indeed, it is well-established that in order for a reference to serve as prior art, it must demonstrate that the claimed invention was in the possession of the public as dictated by the patent statute or case law, including containing a sufficient description of, and an enabling disclosure for, the claimed invention. The reference must contain sufficient technical information to describe the claimed invention to a person of ordinary skill in the art to which the claimed invention pertains and to enable such a person to make and use the claimed subject matter, without requiring undue experimentation. Hillier fails to satisfy these requirements.

The Examiner has addressed Applicants' arguments by citing In re Spada, stating that a chemical composition and it's properties are inseparable. However, the Examiner does not address Applicants' arguments by stating how Hillier is a sufficient prior art reference (i.e., has utility and includes adequate written description and an enabling disclosure) and meets the legal requirements (stated above) necessary to be anticipatory under Section 102.

With respect to In re Spada Applicants respectfully submit that this case is not on point. The present invention does not concern a situation where a known compound is being claimed by including functional language to properties which were previously not appreciated. In such a case the claimed compound is anticipated by the known compound as the known compound necessarily has those same properties. However, the known compound must have utility, be described and enabled in order to anticipate the claimed invention. In the present case, Hillier merely sets forth a sequence of no known utility, for which there is no written description of the sequence and for which no enabling disclosure is provided.

Accordingly, Hillier is nothing more than a sequence of nucleotide bases about which nothing is known. It is no more relevant than would be a randomly computer-generated sequence of nucleotide bases that coincidentally have the same sequence as the claimed invention.

For these reasons, Applicants respectfully submit that withdrawal of the rejections under Section 102 is appropriate and withdrawal thereof is respectfully requested.

Section 103 Rejections

The Examiner has rejected Claims 1, 56 and 58 under 35 U.S.C. 103(a) as unpatentable over the '088 publication in view of Adams et al. for the same reasons set forth in the Office Action dated June 5, 2002. This rejection is respectfully traversed.

In view of the corrected Rule 131 Declaration submitted herewith, the '088 publication is not prior art to the present invention. Accordingly, it cannot be combined with any other reference in making a rejection under Section 103. For this reason, Applicants respectfully submit that this rejection under Section 103 is obviated and withdrawal thereof is respectfully requested.

The Examiner has rejected Claims 59-60 under 35 U.S.C. 103(a) as unpatentable over the '088 publication in view of U.S. Patent No. 6,134,002 for the same reasons set forth in the Office Action dated June 5, 2002. This rejection is respectfully traversed.

In view of the corrected Rule 131 Declaration submitted herewith, the '088 publication is not prior art to the present invention. Accordingly, it cannot be combined with any other reference in

making a rejection under Section 103. For this reason, Applicants respectfully submit that this rejection under Section 103 is obviated and withdrawal thereof is respectfully requested.

The Examiner has rejected Claims 61-64 and 65 under 35 U.S.C. 103(a) as unpatentable over the '088 publication in view of Darnell et al. for the same reasons set forth in the Office Action dated June 5, 2002. This rejection is respectfully traversed.

In view of the corrected Rule 131 Declaration submitted herewith, the '088 publication is not prior art to the present invention. Accordingly, it cannot be combined with any other reference in making a rejection under Section 103. For this reason, Applicants respectfully submit that this rejection under Section 103 is obviated and withdrawal thereof is respectfully requested.

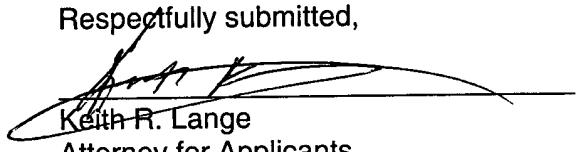
Conclusion

In view of the remarks made herein, Applicants respectfully submit that the claims are in condition for allowance and favorable action is, therefore, respectfully requested.

Please direct any questions concerning this Response or any aspect of this case to the undersigned attorney.

The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment, to Account No. 19-3880 in the name of Bristol-Myers Squibb Company.

Respectfully submitted,



Keith R. Lange
Attorney for Applicants
Reg. No. 44,201

Bristol-Myers Squibb Company
Patent Department
P.O. Box 4000
Princeton, NJ 08543-4000
(609) 252-3218

Date: August 20, 2003

APPENDIX A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN THE APPLICATION OF
STARLING ET AL.
APPLICATION NO: 09/745,605
FILED: DECEMBER 22, 2000
FOR: NOVEL IMMUNOGLOBULIN SUPERFAMILY MEMBERS OF APEX-1, APEX-2 AND APEX-3 AND USES THEREOF

Art Unit: 1644
Examiner: Haddad, Maher M.

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF PRIOR INVENTION IN THE UNITED STATES TO OVERCOME A REFERENCE UNDER 37 C.F.R. § 1.131

Sir:

1. We, Gary C. Starling and Joshua N. Finger, respectively citizens of New Zealand and the United States, residing respectively at 52 James Vincent Drive, Clinton, CT 06413 and 538 Via Dell Caballo, San Marcos, CA 92078 are joint inventors of the above-identified application.

2. At the time of the invention thereof we were working for Bristol-Myers Squibb Company, assignee of the present application. We submit this declaration to establish completion of the invention set forth in this application in the United States at a date prior to December 9, 1999, i.e. the publication date of WO 99/63088 to Baker et al. (hereinafter the '088 publication), which was cited by the Examiner in Office Actions mailed June 5, 2002 and February 25, 2003.

3. From the documents submitted herewith and as set forth hereinbelow, it can be seen that the invention was completed in the United States before December 9, 1999, the publication date of the '088 publication. Completion of the invention prior to December 9, 1999 is shown by

conception and actual reduction to practice of the invention as evidenced by the cloning and sequencing of the APEX-1 gene (hereinafter "APEX-1"), which is also referred to in Exhibit A as DCS4.

4. To establish conception and reduction to practice, i.e. completion of the invention at a date prior to December 9, 1999, the following documents are submitted as evidence:

a. Bristol-Myers Squibb Notebook No. 42973 assigned and completed prior to December 9, 1999 (Exhibit A), pages 42973-103 through 42973-106, 42973-112 through 42973-114, 42973-127 through 42973-129 and page 42973-158. These pages show the full-length cloning of APEX-1 and set forth the nucleotide and amino acid sequences of APEX-1, which correspond to SEQ ID NOS. 1 and 4, respectively, in the present application. The full length cDNA sequence and amino acid translation of APEX-1 is shown on page 42973-158. These notebook records evidence conception and actual reduction to practice of the complete invention prior to December 9, 1999.

5. The materials submitted herewith establish that the invention as claimed was completed, i.e. conceived and reduced to practice, at a date prior to December 9, 1999, the publication date of the '088 publication.

6. This declaration is submitted in a response to a Final Office Action dated February 25, 2003 and is therefore believed to be timely filed.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

DATED:_____

Gary C. Starling

DATED: 08-18-03

Joshua N. Finger

EXHIBIT A

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

BRISTOL-MYERS SQUIBB

NOTEBOOK Nº 42973

Assigned to Joshua N. Jaeger

Department Name _____

Department Number _____

Date Assigned _____

Date Completed _____

Pages Completed from 001 to 200

Continued from Notebook Number _____

Continued in Notebook Number _____

This notebook cannot be transferred to another person

TABLE OF CONTENTS

PROJECT OR EXPERIMENT NO.	• PRODUCT OR SUBSTANCE	STUDY PERFORMED OR OBJECTIVE	P
		Construction of Dendritic substract pole	0
		Hybridization of Southern	09
		Colonies for Screening - Mouse	0
		"	0
		cDNA synthesis	0
		Adapter ligation	09
		Dendritic clones	09
		"	09
		"	09
		Describe hybridization	09
		"	1
		"	1
		BLANK	1
DCS4 (Apex 1)		DCS4 (Clone #4) and Clone # B2 isolation	10
		"	1
		"	10
		~~BLANK~~ Southern blot	10
		BLANK	10
		BLANK	1
		RNA isolation, THP1, NIH3T3, RSA8	1
		Northern hybrid of cell lines	1
		"	1
		DCS4 3'-RACE	1
		"	1
		"	1
		Northern blot of cell lines	1
		DCS4 3'-RACE	1
		"	1
		DCS4 northern analysis - MDN's	1

• USE THE MOST APPROPRIATE DESCRIPTION, E.G.: COMPOUND NUMBER, PRODUCT NAME AND LOT NUMBER, GENERIC NAME, CHEMICAL NAME, CULTURE NUMBER.

TABLE OF CONTENTS

* USE THE MOST APPROPRIATE DESCRIPTION, E.G.: COMPOUND NUMBER, PRODUCT NAME AND LOT NUMBER, GENERIC NAME, CHEMICAL NAME, CULTURE NUMBER.

DATE _____ PROJ. NO. _____ EXPT. NO. _____

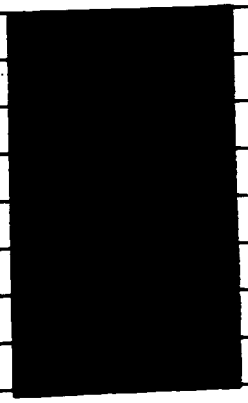
SUBJECT Isolation of Clone #4 and Clone #82

PURPOSE: To isolate the cDNA inserts from clone #4 and clone #82 from plasmid DNA for use as probe.

Materials and Methods

a) Clone #4		85 µl
	React III	01 µl
	EcoRI	1 µl
	dH_2O	3 µl
		10 µl
b) Clone #82		19 µl
	React III	3 µl
	PstI	1.5 µl
	HindIII	1.5 µl
	dH_2O	5.0 µl
		30.0 µl

Results:



Lane 1: λØ Marker
Lane 2: Clone #4
Lane 3: ~~Clone~~ BLANK LANE
Lane 4 BLANK LANE
Lane 5 Clone #82
Lane 6 Clone #82

Discussion: Clone #4 must have a mutated EcoRI site, cut again with HindIII and PstI. Go ahead and isolate clone #82 insert and analyze.

END

SIGNED _____ DATE _____ WITNESSED AND UNDERSTOOD BY: _____ DATE _____

CROSS-REFERENCES:

DATE _____ J. NO _____ EXPT NO _____

SUBJECT: Redigest of Clone #4 using PstI or HindIII

Purpose To drop out insert of clone #4 using enzymes other than EcoRI

Materials and Methods

Clone #4	1.5 ul
Buf II	3 ul
PstI	1.5 ul
HindIII	1.5 ul
dH2O	9.0 ul
	30.0 ul

Results.

Lane 1 Ø marker

Lane 2 H/P digest of DSS4

Lane 3 H/P digest of DSS4

[redacted] ← Gel purify this fragment

← 2nd fragment

Discussion

Just as I suspected DSS4 clone has a mutated EcoRI site as shown by inability of EcoRI to cut out insert (42973-105, Lane 2). However, HindIII and PstI dropped out two fragments. One fragment approximately 400 bp in size will be gel purified and used as a probe (HP400). The 2nd fragment is approximately 80-90 bp and will go into the freezer.

END

SIGNED _____ DATE _____ WITNESSED AND UNDERSTOOD BY: _____ DATE _____

CROSS REFERENCES:

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

DATE ____ ACC. NO. ____ EXPT. NO. ____

SUBJECT Gel purification of clones #5 and clone #4

PURPOSE To gel purify both clones isolated from Detroit cell extraction library by gel extraction in further expt.

MATERIALS & METHODS

See protocol from QIAquick Spin Handbook "QIAquick gel extraction kit" (QIAGEN Cat # 28704)

RESULTS

	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ml	Nucleic Acid ug/ml
1)	0.0048	0.0027	0.0002	1.8457	0.5418	0.0025	23.1278
2)	0.0109	0.0045	-0.0006	2.2434	0.4457	0.0051	57.5225
3)	0.3912	0.2485	0.0023	1.5793	0.6332	0.2463	1944.6359
4)	0.4535	0.2929	0.0039	1.5558	0.6428	0.2890	2248.1201

$[\,] = (A_{260} \times (50\ ^{ug}/_{ml}) \times D) / 1000 = \ ^{ug}/_{ul}$

1) DSS4

$(0.0048 \times 50\ ^{ug}/_{ml} \times 25)/1000 = \boxed{6\ ^{ng}/_{ul}}$

2) DSS8Z

$(0.0109 \times 50\ ^{ug}/_{ml} \times 25)/1000 = \boxed{13\ ^{ng}/_{ul}}$

3) DSS4.1 (MALDEEP DSS4)

$(0.3912 \times 50\ ^{ug}/_{ml} \times 200)/1000 = \boxed{3.9\ ^{ug}/_{ul}}$

4) DSS4.2

$(0.4535 \times 50\ ^{ug}/_{ml} \times 200)/1000 = \boxed{4.5\ ^{ug}/_{ul}}$

DISCUSSION: NONE

END

SIGNED John M. Jin DATE

WITNESSED AND UNDERSTOOD BY: Hasle DATE

CROSS REFERENCES:

DATE ____ NO. ____ EXPT NO. ____

SUBJECT Southern Hybridization to confirm expression of DSS4 in subtracted library

PURPOSE: To confirm the presence of DSS4 in DI2 cDNA, THP1 cDNA and DI2-THP1 subtracted cDNA. To also see if other housekeeping genes such as G3PDH were eliminated in the subtracted cDNA library.

Materials and Methods:

10ug of each cDNA was loaded into each well and ran @ 70Volts for 3 hours. DNA was stained (see gel photo)

	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ml	Nucleic Acid ug/ml
1)	0.6093	0.4039	0.0198	1.5350	0.6515	0.3840	2947.4448
2)	0.9419	0.5982	0.0306	1.6055	0.6229	0.5676	4556.4517
3)	0.2023	0.1188	0.0005	1.7056	0.5863	0.1183	1008.9186

[ ] = Abs × 50 ug/ml × D = ug/ml

1) DI2 cDNA:

$0.4039 \times 50\ ug/ml \times 50 = 1.5\ mg/L$

2) THP1 cDNA: $0.9419 \times 50\ ug/ml \times 50 = 2.4\ mg/L$

3) DI2-THP1 subtracted cDNA $0.2023 \times 50\ ug/ml \times 50 =$ 0.5 ug/l

[redacted]

Lane 1: DI2 cDNA
Lane 2: THP1 cDNA
Lane 3: DI2-THP1 subtracted cDNA

SIGNED ____ DATE ____ WITNESSED AND UNDERSTOOD BY: ____ DATE ____

CROSS REFERENCES:

DATE: ROLL NO. EXPT. NO.

SUBJECT 3' RACE using DSS4 gene specific primer and GT, GT, GT

Purpose: To amplify the remaining 3' end of the unknown DSS4 gene which shows homology to CD84

Materials and Methods

1) cDNA synthesis in Daudi, THP1 and Jurkat cells

poly A+ RNA	2 ul
Primer Q-(dT)17	1 ul
dH2O (DEPC)	9 ul
	12 ul

*Incubate @ 70°C for 10 min Ice 1 min

Add to RNA/primer mix:

10x PCR Buffer	2 ul
25mM MgCl2	1 ul
10 mM dNTP's	2 ul
0.1 M DTT	2 ul
	7 ul

Incubate @ 42°C 5min

Add 1 ul Superscript II RT

Incubate @ 42°C 1 hour

Stop Rxn by 70°C incubation

Add 1 ul RNase H

Store @ -20°C

continued 113

DO NOT WRITE IN THIS MARGIN

SIGNED DATE

WITNESSED AND UNDERSTOOD BY: DATE

CROSS REFERENCES:

DATE. ________ PROJ. NO. ________ EXPT. NO. ________

SUBJECT Continued from 42973-112

continuation from 42973-112

2) Primary PCR using JNF1 and Qo(JNF4)

JNF1	1 µl
JNF4	1 µl
dNTP's	1 µl
10x PCR Buffer	5 µl
~~T~~ 5S cDNA	1 µl
dH_2O	39 µl
50x cDNA Advantage polymerase	1 µl
	50 µl

PCR parameters: 94°C 1 min 1 cycle

94°C 1 min
57°C 1 min } 25 cycles
72°C 2 min

72°C 2 min 1 cycle

3) Secondary PCR using JNF2 and Q- (JNF5)

JNF2	1 µl
JNF5	1 µl
dNTPs	1 µl
10x PCR Buffer	5 µl
1/50 dilution of 1° PCR	1 µl
dH_2O	39 µl
50x Polymerase mix	1 µl
	50 µl

PCR parameters: Same as Primary PCR

Continue to 114

SIGNED [signature] DATE ________ WITNESSED AND UNDERSTOOD BY: [signature] DATE

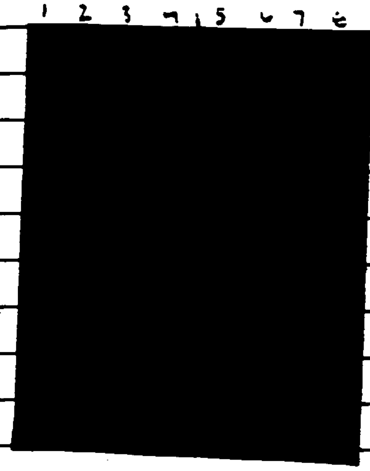
CROSS REFERENCES:

DATE. / /   PROJ. NO.   EXPT

SUBJECT Control for 42973-113

RESULTS:

1 2 3 4 5 6 7 8



Lane 1 ϕX Marker

Lane 2 Dendritic 4-1

Lane 3 THP1 4-1

Lane 4 Jurkat 4-1

Lane 5 Dendritic 2-5

Lane 6 THP1 2-5

Lane 7 Jurkat 2-5

Lane 8 50bp Marker

DISCUSSION:

Lanes 2,3,4 are primary PCR using gsp1 (JNF1) and primer Q0 (JNF4). There seems to be background problems. Either extension time of 2 min was too long or Tm of annealing was low. Secondary PCR using gsp2 (JNF2 and primer Q1 (JNF5) reduced background considerably, but contains several bands which may be nonspecific (lanes 5,6,7). Continue with RACE experiments, starting with 1/50 dilution of 1° PCR, but increase Tm to 58°C and reduce extension to 1 min (since most prominent band in Jurkat line is around 900 bp).

END

SIGNED [signature]   DATE / /

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY [signature] Hasek   DATE / /

DATE: PROJ. EXPT. NO.

SUBJECT: Cloning of Full length DCS4 cDNA including 3'-UTR.

Purpose: To isolate the full length DCS4 cDNA of size approximately 2.6 kb from 50 ul of PCR mixture

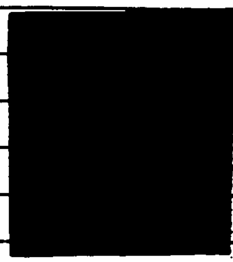
Materials and Methods

PCR		PCR parameters	
FP ([illegible])	2.5 ul	94°C	1 min
RP (TNF4)	1.0 ul	94°C	1 min
dNTP's	1.0 ul	57°C	1 min (30)
10x PCR buffer	5.0 ul	72°C	2.5 min
Advantage Polymerase	1.0 ul	72°C	1.0 min
dH2O	38.5 ul		
Dendritic cDNA	1.0 ul		
	50.0 ul		

Run all 50 ul on 1.2% agarose gel.

Isolate 2.5 kb band using gel extraction kit (QIAgen)

Run on 2.0% gel; check conc.



Lane 1: 1Ø marker

Lane 2: 1 ul isolated PCR fragment

Lane 3: 3 ul isolated PCR fragment

Lane 4: 2 ul Ø marker (1.3 kb band = 16 ng)

Lane 5: 4 ul Ø marker (1.3 kb band = 32 ng)

Conc. of isolated fragment is low: ≈ 2 ng/ul

Ligate fragment into TA cloning vector

Results: Amount of 2.5 kb fragment isolated is very low, but I ligated any just to try and isolate a clone.

Discussion: No discussion yet, wait until transformation.

SIGNED [signature] DATE

WITNESSED AND UNDERSTOOD BY: [signature] DATE

CROSS REFERENCES:

DATE: PROJ. NO. EXPT. NO.

SUBJECT: RT-PCR using primers TNF6 and TNF7

Purpose: To optimize parameters for RT-PCR expts, so as to get one band at 600 bp expected in cell lines which express the DC34 gene.

Materials and Methods

1) 1st PCR:

TNF6 primer	1µl	cDNA's used:
TNF 7 primer	1µl	a) Dendritic
dNTP's	1µl	b) LPS Neut
10x PCR Buffer	5µl	c) THP1
Advantage Polymerase	1µl	
dH_2O	40µl	
DNA	1µl	
	50µl	

Parameters: 94°C 1 min
94°C 1 min
61°C 1 min } 30 cycles
72°C 45 sec
72°C 1 min

Lane 1: λ/Φ marker
Lane 2: Dendritic
Lane 3: LPS Neut
Lane 4: THP1

RESULT 1: 600 bp band was evident, but a high background was present. To continue Redo PCR with elevation of annealing temp to 63°C, in order to reduce background.

2) 2nd PCR:

TNF 6 primer	0.5µl	cDNA's used:
TNF 7 primer	0.5µl	a) LPS NMT ~~Dendritic~~
dNTP's	0.5µl	b) THP1
10x PCR Buffer	2.5µl	
Advantage Polymerase	0.5µl	
dH_2O	20.0µl	
cDNA	0.5µl	
	25.0µl	

Parameters: 94°C 1 min
94°C 1 min
63°C 1 min } 30 cycles
72°C 45 sec
72°C 1 min

Continue 42973-124

SIGNED [signature] DATE

WITNESSED AND UNDERSTOOD BY: [signature] DATE

CROSS REFERENCES:

DATE. PROJ. EXPT. NO.

SUBJECT Continued 42973-128

Result 2: Significant reduction of background, providing conclusive identification of [illegible] band in LPS Non T cells and no band in THP1 as found earlier on page 42973.125.

Discussion: This expt. was done to optimize PCR parameters for RT-PCR of immunological cell lines in order to identify which cell type express the DCS4 transcript. As shown above, LPS Non T cells express DCS4, but THP1 does not. The significance of lack of expression in THP1 mRNA shows the original subtraction expt. [illegible] cDNA's expressed differentially between GMCSF/IL4 differentiated human monocytes and THP1 monocytes. This is also proven by expression of DCS4 in dendritic cDNA. Repeat PCR for all available cell lines.

END

SIGNED [signature] DATE

WITNESSED AND UNDERSTOOD BY: [signature] Halle DATE

CROSS REFERENCES:

DATE. _____ JJ. NO. _____ EXPT. NO. _____

SUBJECT Full length cDNA sequence and a.a. translation Co- DC54

```
                                                    GGAAGTGGCTTCATTTCAGTGGCTGACTTCCAGAGAGCAAT   41
ATGGCTGGTTCCCCAACATGCCTCACCCTCATCTATATCCTTTGGCAGCTCACAGGGTCA  101
M  A  G  S  P  T  C  L  T  L  I  Y  I  L  W  Q  L  T  G  S    20

GCAGCCTCTGGACCCGTGAAAGAGCTGGTCGGTTCCGTTGGTGGGGCCGTGACTTTCCCC  161
A  A  S  G  P  V  K  L  V  G  S  V  G  G  A  V  T  F  P    40

CTGAAGTCCAAAGTAAAGCAAGTTGACTCTATTGTCTGGACCTTCAACACAACCCCTCTT  221
L  K  S  K  V  K  Q  V  D  S  I  V  W  T  F  N  T  T  P  L    60

GTCACCATACAGCCAGAAGGGGGCACTATCATAGTGACCCAAAATCGTAATAGGGAGAGA  281
V  T  I  Q  P  E  G  G  T  I  I  V  T  Q  N  R  N  R  E  R    80

GTAGACTTCCCAGATGGAGGCTACTCCCTGAAGCTCAGCAAACTGAAGAAGAATGACTCA  341
V  D  F  P  D  G  G  Y  S  L  K  L  S  K  L  K  K  N  D  S    100

GGGATCTACTATGTGGGGATATACAGCTCATCACTCCAGCAGCCCTCCACCCAGGAGTAC  401
G  I  Y  Y  V  G  I  Y  S  S  S  L  Q  Q  P  S  T  Q  E  Y    120

GTGCTGCATGTCTACGAGCACCTGTCAAAGCCTAAAGTCACCATGGGTCTGCAGAGCAAT  461
V  L  H  V  Y  E  H  L  S  K  P  K  V  T  M  G  L  Q  S  N    140

AAGAATGGCACCTGTGTGACCAATCTGACATGCTGCATGGAACATGGGGAAGAGGATGTG  521
K  N  G  T  C  V  T  N  L  T  C  C  M  E  H  G  E  E  D  V    160

ATTTATACCTGGAAGGCCCTGGGGCAAGCAGCCAATGAGTCCCATAATGGGTCCATCCTC  581
I  Y  T  W  K  A  L  G  Q  A  A  N  E  S  H  N  G  S  I  L    180

CCCATCTCCTGGAGATGGGGAGAAAGTGATATGACCTTCATCTGCGTTGCCAGGAACCCT  641
P  I  S  W  R  W  G  E  S  D  M  T  F  I  C  V  A  R  N  P    200

GTCAGCAGAAACTTCTCAAGCCCCATCCTTGCCAGGAAGCTCTGTGAAGGTGCTGCTGAT  701
V  S  R  N  F  L  K  P  S  P  I  L  A  R  K  L  C  E  G  A  A  D    220

GACCCAGATTCCTCCATGGTCCTCCTGTGTCTCCTGTTGGTGCCCCTCCTGCTCAGTCTC  761
D  P  D  S  S  M  V  L  L  C  L  L  L  V  P  L  L  L  S  L    240

TTTGTACTGGGGCTATTTCTTTGGTTTCTGAAGAGAGAGAGACAAGAAGAGTACATTGAA  821
F  V  L  G  L  F  L  W  F  L  K  R  E  R  Q  E  E  Y  I  E    260

GAGAAGAAGAGAGTGGACATTTGTCGGGAAACTCCTAACATATGCCCCCATTCTGGAGAG  881
E  K  K  R  V  D  I  C  R  E  T  P  N  I  C  P  H  S  G  E    280

AACACAGAGTACGACACAATCCCTCACACTAATAGAACAATCCTAAAGGAAGATCCAGCA  941
N  T  E  Y  D  T  I  P  H  T  N  R  T  I  L  K  E  D  P  A    300

AATACGGTTTACTCCACTGTGGAAATACCGAAAAAGATGGAAAATCCCCACTCACTGCTC 1001
N  T  V  Y  S  T  V  E  I  P  K  K  M  E  N  P  H  S  L  L    320

ACGATGCCAGACACACCAAGGCTATTTGCCTATGAGAATGTTATCTAGACAGCAGTGCAC 1061
T  M  P  D  T  P  R  L  F  A  Y  E  N  V  I  *                335

TCCCCTAAGTCTCTGCTCAAAAAAAAAACAATTCTCGGCCCAAAGAAAACAATCAGAAGA 1121
ATTCACTGATTTGACTAGAAACATCAAGGAAGAATGAAGAACGTTGACTTTTTTCCAGGA 1181
TAAATTATCTCTGATGCTTCTTTAGATTTAAGAGTTCGTAATTCCATCCACTGCTGAGAA 1241
ATCTCCTCAAACCCAGAAGGTTTAATCACTTCATCCCAAAAATGGGATTGTGAATGTCAG 1301
CAAACCATAAAAAAAAGTGCTTAGAAGTATTCCTATAGAAATGTAAATGCAAGGTCACACA 1361
TATTAATGACAGCCTGTTGTATTAATGATGGCTCCAGGTCAGTGTCTGGAGTTTCATTCC 1421
ATCCCAGGGCTTGGATGTCAGGATTATACCAAGAGTCTTGCTACCAGGAGGGCAAGAAGA 1481
CCAAAACAGACAGACAAGTCCAGCAGAAGCAGATGCACCTGACAAAAATGGATGTATTAA 1541
TTGGCTCTATAAACTATGTGCCCAGCACTATGCTGAGCTTACACTAATTGGTCAGACGTG 1601
CTGTCTGCCCTCATGAAATTGGCTCCAAATGAATGAACTACTTTCATGAGCAGTTGTAGC 1661
AGGCCTGACCACAGATTCCCAGAGGGCCAGGTGTGGATCCACAGGACTTGAAGGTCAAAG 1721
TTCACAAAGATGAAGAATCAGGGTAGCTGACCATGTTTGGCAGATACTATAATGGAGACA 1781
CAGAAGTGTGCATGGCCCAAGGACAAGGACCTCCAGCCAGGCTTCATTTATGCACTTGTG 1841
CTGCAAAAGAAAAGTCTAGGTTTTAAGGCTGTGCCAGAACCCATCCCAATAAAGAGACCG 1901
AGTCTGAAGTCACATTGTAAATCTAGTGTAGGAGACTTGGAGTCAGGCAGTGAGACTGGT 1961
GGGGCACGGGGGGCAGTGGGTACTTGTAAACCTTTAAAGATGGTTAATTCATTCAATAGA 2021
TATTTATTAAGAACCTACTATGCGGCCCGGCATGGTGGCTCACACCTGTAATCCCAGCAC 2081
TTTGGGAGGCCAAGGTGGGTGGGTCATCTGAGGTCAGGAGTTCAAGACCAGCCTGGCCAA 2141
CATGGTGAAACCCCATCTCTACTAAAGATCAAAATTTGCTGAGCGTGGTGGTGTGCACCT 2201
GTATCCCAGCTACTCGAGAGGCCAAGGCATGAGAATCGCTTGAACCTGGAGGTGAGGTTG 2261
CAGTGAGCTGAGATGGCACCACTGCACTCCGGCCTAGGCAACGAGAGCAAAACTCCAATA 2321
CAAACAAACAAACAAACACCTGTGCTAGGTCAGTCTGGCACGTAAGATGAACATCCCTAC 2381
CAACACAGAGCTCACCATCTCTTATACTTAAGTGAAAAACATGGGGAAGGGGAAAGGGGA 2441
ATGGCTGCTTTTGATATGTTCCCTGACGCATATCTTGAATGGAGACCTCCCTACCAAGTG 2501
ATGAAAGTGTTGAAAAACTTAATAACAAATGCTTGTTGGGCAAGAATGGGATTGAGGATT 2561
ATCTTCTCTCAGAAAGGCATTGTGAAGGAATTGAGCCAGATCTCTCTCCCTACTGCAAAA 2621
CCCTATTGTAGTAAAAAAGTCTTCTTTACTATCTTAATAAAACAGATATTGTGAGATTCA 2681
CATAAAAAAAAAAAAAAAAAAAA
```

Word Document
FLDC54.doc

SIGNED _____ DATE _____

CROSS REFERENCES:

WITNESSED AND UNDERSTOOD BY: _____ DATE _____

APPENDIX B

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF
STARLING ET AL.
APPLICATION NO: 09/745,605
FILED: DECEMBER 22, 2000
FOR: NOVEL IMMUNOGLOBULIN SUPERFAMILY MEMBERS OF APEX-1, APEX-2 AND APEX-3 AND USES THEREOF

Art Unit: 1644
Examiner: Haddad, Maher M.

Assistant Commissioner for Patents
Washington, D.C. 20231

DECLARATION OF PRIOR INVENTION IN THE UNITED STATES TO OVERCOME A REFERENCE UNDER 37 C.F.R. § 1.131

Sir:

1. We, Gary C. Starling and Joshua N. Finger, respectively citizens of New Zealand and the United States, residing respectively at 52 James Vincent Drive, Clinton, CT 06413 and 538 Via Dell Caballo, San Marcos, CA 92078 are joint inventors of the above-identified application.

2. At the time of the invention thereof we were working for Bristol-Myers Squibb Company, assignee of the present application. We submit this declaration to establish completion of the invention set forth in this application in the United States at a date prior to December 9, 1999, i.e. the publication date of WO 99/63088 to Baker et al. (hereinafter the '088 publication), which was cited by the Examiner in Office Actions mailed June 5, 2002 and February 25, 2003.

3. From the documents submitted herewith and as set forth hereinbelow, it can be seen that the invention was completed in the United States before December 9, 1999, the publication date of the '088 publication. Completion of the invention prior to December 9, 1999 is shown by

conception and actual reduction to practice of the invention as evidenced by the cloning and sequencing of the APEX-1 gene (hereinafter "APEX-1"), which is also referred to in Exhibit A as DCS4.

4. To establish conception and reduction to practice, i.e. completion of the invention at a date prior to December 9, 1999, the following documents are submitted as evidence:

a. Bristol-Myers Squibb Notebook No. 42973 assigned and completed prior to December 9, 1999 (Exhibit A), pages 42973-103 through 42973-106, 42973-112 through 42973-114, 42973-127 through 42973-129 and page 42973-158. These pages show the full-length cloning of APEX-1 and set forth the nucleotide and amino acid sequences of APEX-1, which correspond to SEQ ID NOS. 1 and 4, respectively, in the present application. The full length cDNA sequence and amino acid translation of APEX-1 is shown on page 42973-158. These notebook records evidence conception and actual reduction to practice of the complete invention prior to December 9, 1999.

5. The materials submitted herewith establish that the invention as claimed was completed, i.e. conceived and reduced to practice, at a date prior to December 9, 1999, the publication date of the '088 publication.

6. This declaration is submitted in a response to a Final Office Action dated February 25, 2003 and is therefore believed to be timely filed.

7. We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information or belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and like so made are punishable by fine or imprisonment or both under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

DATED: 18 August 2003 Gary C. Starling

DATED: _____ Joshua N. Finger

EXHIBIT A

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

BRISTOL-MYERS SQUIBB

NOTEBOOK Nº 42973

Assigned to Joshua N. Jaeger

Department Name _____

Department Number _____

Date Assigned _____

Date Completed _____

Pages Completed from 001 to 200

Continued from Notebook Number _____

Continued in Notebook Number _____

This notebook cannot be transferred to another person

TABLE OF CONTENTS

• USE THE MOST APPROPRIATE DESCRIPTION, E.G.: COMPOUND NUMBER, PRODUCT NAME AND LOT NUMBER, GENERIC NAME, CHEMICAL NAME, CULTURE NUMBER.

TABLE OF CONTENTS

* USE THE MOST APPROPRIATE DESCRIPTION, E.G.: COMPOUND NUMBER, PRODUCT NAME AND LOT NUMBER, GENERIC NAME, CHEMICAL NAME, CULTURE NUMBER.

DATE _____ PROJ. NO. _____ EXPT. NO. _____

SUBJECT Isolation of Clone #4 and Clone #82

PURPOSE: To isolate the cDNA inserts from clone #4 and clone #82 from plasmid DNA for use as a probe.

Materials and Methods

a) Clone #4		0.5 ml
React III		0.1 ml
EcoRI		1 ml
dH_2O		3 ml
		10 ml

b) Clone #82	19 ml
React III	3 ml
PstI	1.5 ml
HindIII	1.5 ml
dH_2O	5.0 ml
	30.0 ml

Results:

[redacted]

Lane 1: λØ Marker
Lane 2: Clone #4
Lane 3: ~~Clone~~ BLANK LANE
Lane 4 BLANK LANE
Lane 5 Clone #82
Lane 6 Clone #82

Discussion: Clone #4 must have a mutated EcoRI site, cut again with HindIII and PstI. Go ahead and isolate clone #82 insert and analyze.

END

SIGNED _____ DATE _____ WITNESSED AND UNDERSTOOD BY: _____ DATE _____

CROSS REFERENCES:

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

DATE _____ J. NO _____ EXPT NO _____

SUBJECT: Redigest of Clone #4 using Pst I & Hind III

Purpose To drop out insert of clone #4 using enzymes other than EcoRI

Materials and Methods

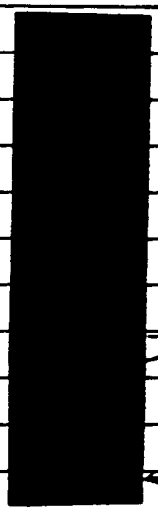
Clone #4	1.5 ul
Buff II	3 ul
Pst I	1.5 ul
Hind III	1.5 ul
dH_2O	9.0 ul
	30.0 ul

Results.

Lane 1 Ø marker

Lane 2 HIP digest of DSS4

Lane 3 HIP digest of DSS4



← Gel purify this fragment

← 2nd fragment

Discussion

Just as I suspected DSS4 clone has a mutated EcoRI site as shown by inability of EcoRI to cut out insert (42973-103, Lane 2). However, Hind III and Pst I dropped out two fragments. One fragment approximately 400 bp in size will be gel purified and used as a probe (HP400). The 2nd fragment is approximately 80-90 bp and will go into the trash.

END

SIGNED _____ DATE _____ WITNESSED AND UNDERSTOOD BY: _____ DATE _____

CROSS REFERENCES:

DATE. — ACL NO. EXPT. NO.

SUBJECT Gel purification of Clone #51 and Clone #4

PURPOSE To gel purify both clones isolated from [illegible] cell [illegible] library to be used as probes in further expt.

Materials & Methods

See protocol from QIAquick Spin Handbook "QIAquick gel extraction kit" (QIAGEN Cat # 28704)

RESULTS

	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ml	Nucleic Acid ug/ml
1)	0.0048	0.0027	0.0002	1.8457	0.5418	0.0025	23.1278
2)	0.0109	0.0045	-0.0006	2.2434	0.4457	0.0051	57.5225
3)	0.3912	0.2485	0.0023	1.5793	0.6332	0.2463	1944.6359
4)	0.4535	0.2929	0.0039	1.5558	0.6428	0.2890	2248.1201

$[\ ] = (A_{260} \times (50\ \mu g/ml) \times D)/1000 = \mu g/\mu l$

1) DSS4
$(0.0048 \times 50\ \mu g/ml \times 25)/1000 = 6\ ng/\mu l$

2) DSS82
$(0.0109 \times 50\ \mu g/ml \times 25)/1000 = 13\ ng/\mu l$

Maxiprep DSS4

3) DSS4.1 $(0.3912 \times 50\ \mu g/ml \times 200)/1000 = 3.9\ \mu g/\mu l$

4) DSS4.2 $(0.4535 \times 50\ \mu g/ml \times 200)/1000 = 4.5\ \mu g/\mu l$

DISCUSSION: NONE

END

SIGNED [signature] DATE

WITNESSED AND UNDERSTOOD BY [signature] DATE

CROSS REFERENCES:

BRISTOL-MYERS SQUIBB PHARMACEUTICAL RESEARCH INSTITUTE

DATE. ____ ____ NO. ____ EXPT NO. ____

SUBJECT Southern Hybridization to confirm expression of DSS4 in subtracted library

PURPOSE: To confirm the presence of DSS4 in D12 cDNA, THP1 cDNA and D12-THP1 subtracted cDNA. To also see if other housekeeping genes such as G3PDH were present in the subtracted cDNA libraries.

Materials and Methods:

10ug of each cDNA was loaded into each well and ran @ 70Volts for 3 hours. DNA was stained (ethidium bromide)

	abs 260.0 nm	abs 280.0 nm	bkg abs 320.0 nm	260.0 nm / 280.0 nm	280.0 nm / 260.0 nm	Protein ug/ml	Nucleic Acid ug/ml
1)	0.6093	0.4039	0.0198	1.5350	0.6515	0.3840	2947.4448
2)	0.9419	0.5982	0.0306	1.6055	0.6229	0.5676	4556.4517
3)	0.2023	0.1188	0.0005	1.7056	0.5863	0.1183	1008.9186

[ ] = Abs x 50 ug/ml x D = ug/ml

1) D12 cDNA:

0.4039 x 50 ug/ml x 50 = 1.5 mg/ml

2) THP1 cDNA: 0.9419 x 50 ug/ml x 50 = 2.4 mg/ml

3) D12-THP1 subtracted cDNA 0.2023 x 50 ug/ml x 50 = c.5 ug/ml

[redacted]

AT Lane 1 : D12 cDNA

Lane 2 : THP1 cDNA

Lane 3 : D12-THP1 subtracted cDNA

SIGNED ____ DATE ____ WITNESSED AND UNDERSTOOD BY: ____ DATE ____

CROSS REFERENCES:

BRISTOL-MYERS SQUIBB PHA[RM]ACEUTICAL RESEARCH INSTITUTE

DATE: PROJ. NO. EXPT. NO.

SUBJECT 3' RACE using DS54 gene spec. primer and QT, QI, QO

Purpose: To amplify the remaining 3' end of the unknown DS54 gene which shows homology to CD84

Materials and Methods

1) cDNA synthesis in Dendritic, THP1 and Jurkat cells

poly A+ RNA	2 ul
Primer QT (3NF3)	1 ul
dH2O (DEPC)	9 ul
	12 ul

* Incubate @ 70°C for 10 min Ice 1 min

Add to RNA/primer mix:

10x PCR Buffer	2 ul
25 mM $MgCl_2$	1 ul
10 mM dNTP's	2 ul
0.1 M DTT	2 ul
	7 ul

Incubate @ 42°C 5 min
Add 1 ul Superscript II RT
Incubate @ 42°C 1 hour
Stop Rxn by 70°C incubation
Add 1 ul RNase H
Store @ -20°C

continued 113

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WITNESSED AND UNDERSTOOD BY: [signature] DATE

CROSS REFERENCES:

DATE. PROJ. NO. EXPT. NO.

SUBJECT Combwl for 42973-112

Combination for - 42973-112

2) Primary PCR using INF1 and Q(INF4)

INF1	1 µl
INF4	1 µl
dNTP's	1 µl
10 x PCR Buffer	5 µl
~~Tss~~ cDNA	1 µl
dH_2O	39 µl
50x cDNA Amplification enzyme	1 µl
	50 µl

PCR parameters: 94°C 1min 1cycle
94°C 1min
57°C 1min } 25cycles
72°C 2min
72°C 2min 1cycle

3) Secondary PCR using INF2 and Q-(INF5)

INF2	1 µl
INF5	1 µl
dNTP's	1 µl
10x PCR Buffer	5 µl
1/50 dilution of 1° PCR	1 µl
dH_2O	39 µl
50x Polymerase mix	1 µl
	50 µl

PCR parameters: Same as Primary PCR

Combine to 114

SIGNED DATE WITNESSED AND UNDERSTOOD BY: DATE

CROSS REFERENCES:

DATE. ——

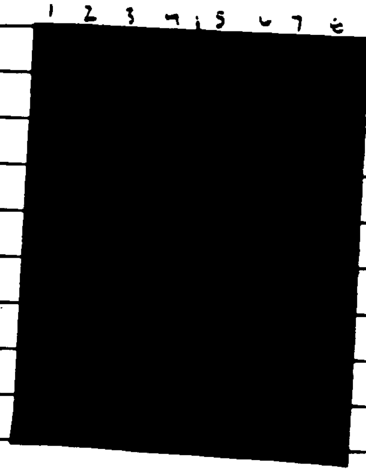
PROJ. NO. ——

EXPT ——

SUBJECT Contract Con 42923-113

Results:

1 2 3 4 5 6 7 8



Lane 1 λ/¥ Marker

Lane 2 Dendritic 4-1

Lane 3 THP1 4-1

Lane 4 Jurkat 4-1

Lane 5 Dendritic 2-5

Lane 6 THP1 2-5

Lane 7 Jurkat 2-5

Lane 8 50bp Marker

DISCUSSION:

Lanes 2,3,4 are primary PCR using gsp1 (JNF1) and primer Q0 (JNF4). There seems to be background problems. Either extension time of 2 min was too long or Tm of annealing was low. Secondary PCR using gsp2 (JNF2) and primer Q1 (JNF5) reduced background considerably, but contains several bands which may be nonspecific (Lanes 5,6,7). Continue with RACE experiments, starting with 1/50 dilution of 1° PCR, but increase Tm to 58°C and reduce extension to 1 min (since most prominent band in Jurkat line is around 900 bp).

END

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DATE

CROSS REFERENCES:

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DATE

DATE: PROJ. EXPT. NO.

SUBJECT Cloning of Full length DC54 cDNA encoding 3'-UTR.

PURPOSE: To isolate the full length DC54 cDNA of size approximately 2.6 kb from 50 ul of PCR mixture

Materials and Methods

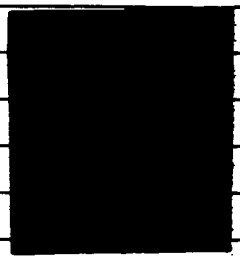
PCR

EP (cloned, pt)	2.5 ul
RP (JNF4)	1.0 ul
dNTP's	1.0 ul
10x PCR Buffer	5.0 ul
Advantage Polymerase	1.0 ul
dH2O	38.5 ul
Denatured cDNA	1.0 ul
	50.0 ul

PCR parameters

94°C 1 min
94°C 1 min
57°C 1 min } 30
72°C 2.5 min
72°C 10 min

Run all 50 ul on 1.2% agarose gel.
Isolate 2.5 kb band using gel extraction kit (QIAgen)
Run on 2.0% gel; check conc.



Lane 1: λ Ø marker.
Lane 2: 1 ul isolated PCR fragment
Lane 3: 3 ul isolated PCR fragment
Lane 4: 2 ul Ø marker (1.5 kb band = 16 ng)
Lane 5: 4 ul Ø marker (1.5 kb band = 32 ng)

Conc. of isolated fragment is 100 : = 2 ng/ul

Ligate fragment into TA cloning vector

Result: Amount of 2.5 kb fragment isolated is very low, but I ligated any just to try and isolate a clone.

Discussion: No discussion yet, wait until transformation.

SIGNED [signature] DATE

WITNESSED AND UNDERSTOOD BY: [signature] DATE

CROSS REFERENCES:

BRISTOL-MYERS SQUIBB F RMACEUTICAL RESEARCH INSTITUTE

DATE. PROJ. NO. EXPT. NO.

SUBJECT RT-PCR using primers TNF6 and TNF7

Purpose: To optimize parameters for RT-PCR expts, so as to get one band at 600 bp amplified in cell lines which express the DCS4 gene

Materials and Methods

1) 1st PCR:

TNF 6 primer	1 µl		cDNA's used:
TNF 7 primer	1 µl		a) Dendritic
dNTP's	1 µl		b) LPS NeuT cell
10x PCR Buffer	5 µl		c) THP1
Advantage Polymerase	1 µl		
dH₂O	40 µl		
DNA	1 µl		
	50 µl		

Parameters: 94°C 1 min
94°C 1 min
61°C 1 min } 30 cycles
72°C 45 sec
72°C 1 min

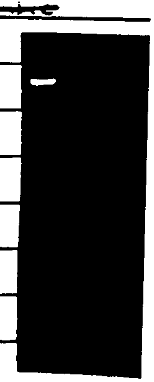
Lane 1: λ/Φ marker
Lane 2: Dendritic
Lane 3: LPS NeuT
Lane 4: THP1

RESULT 1: 600 bp band was evident, but a high background was present. To continue, Redo PCR with elevation of annealing temp to 63°C, in order to reduce background.

2) 2nd PCR:

TNF 6 primer	0.5 µl		cDNA's used:
TNF 7 primer	0.5 µl		a) LPS NMT ~~Dendritic~~
dNTP's	0.5 µl		b) THP1
10x PCR Buffer	2.5 µl		
Advantage Polymerase	0.5 µl		
dH₂O	20.0 µl		
cDNA	0.5 µl		
	25.0 µl		

Parameters: 94°C 1 min
94°C 1 min
63°C 1 min } 30 cycles
72°C 45 sec
72°C 1 min



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CROSS REFERENCES:

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SUBJECT: Continued 42973-128

Result 2: Significant reduction of background, permitting conclusive identification of 400 bp band in LPS Mono T cell and no band in THP1 as found earlier on page 42973-125.

Discussion: This expt. was done to optimize PCR parameters for RT-PCR of immunological cell lines in order to identify which cell type express the OCS4 transcript. As shown above, LPS Mono T cells express OCS4, but THP1 does not. The significance of lack of expression in THP1 mRNA since the original subtraction expt. identified cDNA's expressed differentially between GMCSF/IL4 differentiated human monocytes and THP1 monocytes. This is also proven by expression of OCS4 in dendritic cDNA. Repeat PCR for all available cell lines.

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CROSS REFERENCES:

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SUBJECT Full length cDNA sequence and a.a. translation for DC54

Word Document
FLDC54.doc

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                                                GGAAGTGGCTTCATTTCAGTGGCTGACTTCCAGAGAGCAAT   41
ATGGCTGGTTCCCCAACATGCCTCACCCTCATCTATATCCTTTGGCAGCTCACAGGGTCA  101
M  A  G  S  P  T  C  L  T  L  I  Y  I  L  W  Q  L  T  G  S    20

GCAGCCTCTGGACCCGTGAAAGAGCTGGTCGGTTCCGTTGGTGGGGCCGTGACTTTCCCC  161
A  A  S  G  P  V  K  E  L  V  G  S  V  G  G  A  V  T  F  P    40

CTGAAGTCCAAAGTAAAGCAAGTTGACTCTATTGTCTGGACCTTCAACACAACCCCTCTT  221
L  K  S  K  V  K  Q  V  D  S  I  V  W  T  F  N  T  T  P  L    60

GTCACCATACAGCCAGAAGGGGGCACTATCATAGTGACCCAAAATCGTAATAGGGAGAGA  281
V  T  I  Q  P  E  G  G  T  I  I  V  T  Q  N  R  N  R  E  R    80

GTAGACTTCCCAGATGGAGGCTACTCCCTGAAGCTCAGCAAACTGAAGAAGAATGACTCA  341
V  D  F  P  D  G  G  Y  S  L  K  L  S  K  L  K  K  N  D  S    100

GGGATCTACTATGTGGGGATATACAGCTCATCACTCCAGCAGCCCTCCACCCAGGAGTAC  401
G  I  Y  Y  V  G  I  Y  S  S  S  L  Q  Q  P  S  T  Q  E  Y    120

GTGCTGCATGTCTACGAGCACCTGTCAAAGCCTAAAGTCACCATGGGTCTGCAGAGCAAT  461
V  L  H  V  Y  E  H  L  S  K  P  K  V  T  M  G  L  Q  S  N    140

AAGAATGGCACCTGTGTGACCAATCTGACATGCTGCATGGAACATGGGGAAGAGGATGTG  521
K  N  G  T  C  V  T  N  L  T  C  C  M  E  H  G  E  E  D  V    160

ATTTATACCTGGAAGGCCCTGGGGCAAGCAGCCAATGAGTCCCATAATGGGTCCATCCTC  581
I  Y  T  W  K  A  L  G  Q  A  A  N  E  S  H  N  G  S  I  L    180

CCCATCTCCTGGAGATGGGGAGAAAGTGATATGACCTTCATCTGCGTTGCCAGGAACCCT  641
P  I  S  W  R  W  G  E  S  D  M  T  F  I  C  V  A  R  N  P    200

GTCAGCAGAAACTTCTCAAGCCCCATCCTTGCCAGGAAGCTCTGTGAAGGTGCTGCTGAT  701
V  S  R  N  F  S  S  P  I  L  A  R  K  L  C  E  G  A  A  D    220

GACCCAGATTCCTCCATGGTCCTCCTGTGTCTCCTGTTGGTGCCCCTCCTGCTCAGTCTC  761
D  P  D  S  S  M  V  L  L  C  L  L  L  V  P  L  L  L  S  L    240

TTTGTACTGGGGCTATTTCTTTGGTTTCTGAAGAGAGAGAGACAAGAAGAGTACATTGAA  821
F  V  L  G  L  F  L  W  F  L  K  R  E  R  Q  E  E  Y  I  E    260

GAGAAGAAGAGAGTGGACATTTGTCGGGAAACTCCTAACATATGCCCCCATTCTGGAGAG  881
E  K  K  R  V  D  I  C  R  E  T  P  N  I  C  P  H  S  G  E    280

AACACAGAGTACGACACAATCCCTCACACTAATAGAACAATCCTAAAGGAAGATCCAGCA  941
N  T  E  Y  D  T  I  P  H  T  N  R  T  I  L  K  E  D  P  A    300

AATACGGTTTACTCCACTGTGGAAATACCGAAAAAGATGGAAAATCCCCACTCACTGCTC 1001
N  T  V  Y  S  T  V  E  I  P  K  K  M  E  N  P  H  S  L  L    320

ACGATGCCAGACACACCAAGGCTATTTGCCTATGAGAATGTTATCTAGACAGCAGTGCAC 1061
T  M  P  D  T  P  R  L  F  A  Y  E  N  V  I  *               335

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ATTCACTGATTTGACTAGAAACATCAAGGAAGAATGAAGAACGTTGACTTTTTTCCAGGA 1181
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CAAACCATAAAAAAAAGTGCTTAGAAGTATTCCTATAGAAATGTAAATGCAAGGTCACACA 1361
TATTAATGACAGCCTGTTGTATTAATGATGGCTCCAGGTCAGTGTCTGGAGTTTCATTCC 1421
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CROSS REFERENCES: